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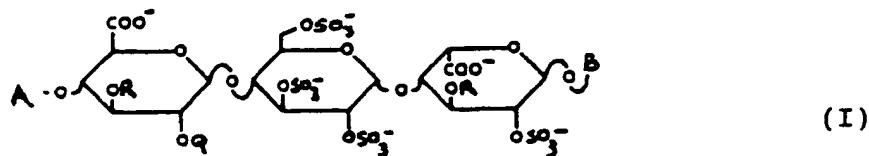
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㉚ Carbohydrate derivatives comprising a trisaccharide unit.

㉛ Carbohydrate derivatives comprising a trisaccharide unit having the general formula I



in which R is alkyl, aryl, or aralkyl;

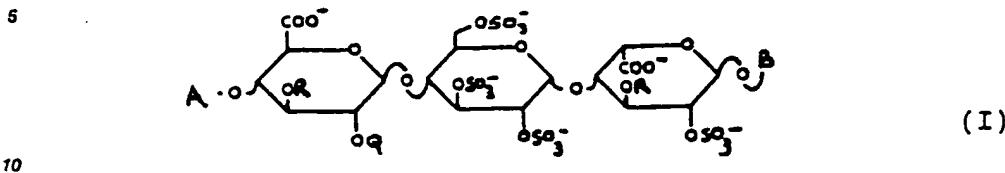
Q is alkyl, aryl, aralkyl, or SO3^-;

A and B are independently selected from hydrogen, alkyl, aryl, aralkyl, or a carbohydrate group;  
and the charged moieties are compensated by counter-ions.

The carbohydrate derivatives of this invention possess anti-thrombotic activity, and especially they have potent anti-Xa activity without activating thrombin via anti-thrombin-III (AT-III) and smooth muscle cell proliferation inhibiting activity.

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The invention concerns a carbohydrate derivative comprising a trisaccharide unit of the general formula



- in which R is alkyl, aryl, or aralkyl;  
 Q is alkyl, aryl, aralkyl, or  $\text{SO}_3^-$ ;  
 A and B are independently selected from hydrogen, alkyl, aryl, aralkyl, or a carbohydrate group;  
 15 and the charged moieties are compensated by counter-ions.

The invention also deals with a process for the preparation of said carbohydrate derivative, as well as with pharmaceutical compositions containing the same.

The carbohydrate derivatives of this invention possess anti-thrombotic activity, and especially they have potent anti- $X_a$  activity without activating thrombin via anti-thrombin-III (AT-III) and they inhibit smooth muscle 20 cell proliferation.

Related carbohydrates are known from European patent application 0,301,618. The compounds described therein are sulfated pentasaccharides DEFGH (letter code according to EP 0,301,618). The trisaccharide unit of the instant compounds corresponds with the EFG part of the prior art compounds, but differs with it, in that free hydroxy groups of the uronic acids E and G are alkylated, arylated, or aralkylated.

25 It has now been found that the compounds of this invention have a better binding affinity to antithrombin III, which results in a better pharmacokinetic profile, longer half-life times, better specificity, and lower therapeutic doses and thus lesser side-effects. Furthermore, the compounds of this invention have a substantially better heparin cofactor II (HCII) mediated antithrombin activity, and are, therefore, more effective as thrombin generation inhibitors than the prior art compounds. The inclusion of alkyl, aryl, or 30 aralkyl functionalized uronic acids gives further a very important synthetic advantage over the prior art compounds. By functionalizing the hydroxy groups with alkyl, aryl, or aralkyl groups, it is in most cases redundant to prepare temporarily protected carbohydrates, which makes the synthetic pathway considerably shorter and simpler, whereas the replacement of the glucosamine unit F by a glucose unit further simplifies the synthesis of the trisaccharide unit significantly. Moreover, an additional advantage of the synthesis of 35 the compounds of the invention is that the nature of the temporarily protective groups, which are necessary for the protection of the hydroxy groups to be sulfated, is not critical. In this respect it is of interest to note that the biological activities of compounds with Q is alkyl, aryl, or aralkyl and with Q is  $\text{SO}_3^-$  are more or less equal, thus making it less critical to use e.g. benzyl or acyl protection on position 2 of the glucuronic acid residue. Because 2-acyl protection of the glucuronic acid facilitates  $\beta$  coupling of this residue to a 40 glucose unit, compounds of formula (I) having Q is  $\text{SO}_3^-$  are frequently preferred.

The alkyl group in the definition of R, Q, A, and B is a branched or unbranched alkyl group having 1-20 carbon atoms. Alkyl groups for different groups R may be different. Examples are methyl, ethyl, isopropyl, butyl, sec-butyl, pentyl, neopentyl, hexyl, octyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, octadecyl, and eicosyl. Preferred are the alkyl groups having 1-6 carbon atoms. More preferred 45 are the alkyl groups having 1-4 carbon atoms, and most preferred is a methyl group. The preferred group Q is methyl or  $\text{SO}_3^-$ .

The term aryl in the definition of R, Q, A, and B means an aromatic group, preferably phenyl, which may be substituted by OH, alkyl having 1-4 carbon atoms, alkoxy having 1-4 carbon atoms, halogen (preferably fluorine, chlorine, or bromine), or NR'R'', wherein R' and R'' are independently selected from 50 hydrogen, alkyl having 1-4 carbon atoms, CF<sub>3</sub>, benzyloxycarbonyl, carboxyl, and  $\text{SO}_3^-$ .

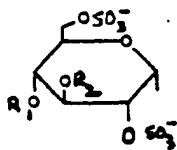
The term aralkyl means an aralkyl group in which the alkyl moiety is an alkyl group having 1-4 carbon atoms and the aryl moiety is an aryl group as previously defined.

The alkyl group having 1-4 carbon atoms is methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, or tert-butyl, and the alkyl moiety of the alkoxy group having 1-4 carbon atoms has the same meaning.

55 The term carbohydrate group in the definition of A and B means a mono- or disaccharide, which optionally may be sulfated, or may be protected by alkyl, aryl, or aralkyl groups as previously defined. Suitable carbohydrate groups are, for instance, free, sulfated, alkylated, arylated, or aralkylated derivatives of glucose, mannose, galactose, glucosamine, galactosamine, glucuronic acid, iduronic acid, xylose,

arabinose or disaccharides thereof. Preferably, A represents a glucose derivative having the structure II:

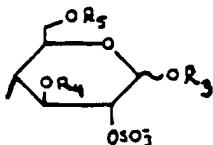
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(II)

10 and B represents a glucose derivative having the structure III:

15



(III)

in which R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> are independently selected from hydrogen, alkyl, aryl, aralkyl, or SO<sub>3</sub><sup>-</sup>, whereas alkyl, aryl, and aralkyl has the previously given meanings, or their derivatives in which the charged moieties are compensated by counter-ions. Very suitable derivatives II and III are those in which R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> are methyl, R<sub>4</sub> and R<sub>5</sub> are SO<sub>3</sub><sup>-</sup>, and OR<sub>3</sub> adopts preferably the  $\alpha$ -configuration. Another suitable group A has structure II in which R<sub>1</sub> is methyl, and R<sub>2</sub> is SO<sub>3</sub><sup>-</sup>, and another suitable group B has structure III in which R<sub>4</sub> and R<sub>5</sub> are SO<sub>3</sub><sup>-</sup>, and R<sub>3</sub> is an alkyl group with 6-20 carbon atoms.

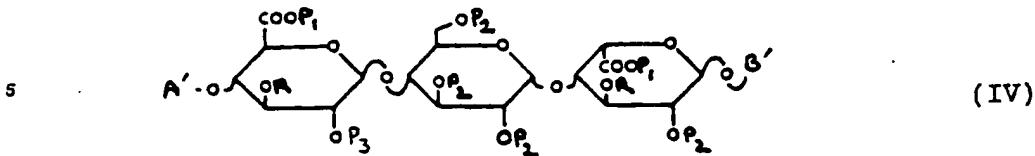
25 It is generally believed that multipoint key polar interactions are of essential importance throughout molecular biology for ensuring high selectivity in noncovalent molecular associations, and that substitution of only one of the key hydroxy groups of an oligosaccharide by a hydrophobic group (and invariably a number of the hydroxy groups prove outstandingly essential to complex formation) can result in complete loss of the affinity by the protein. Remarkably, however, the preferred compounds of this invention having 30 O-alkyl and O-sulfate groups without having free hydroxy groups, still show the full-blown activity.

The counter-ions which compensate the charged moieties are pharmaceutically acceptable counter-ions, like hydrogen, or more preferably alkali or earth-alkali metal ions, like sodium or calcium.

35 The carbohydrates according to this invention may be prepared according to well known methods described and used for the synthesis of polysaccharides. In this respect, particular reference is made to the previously mentioned European patent 0,301,618, in which methods for the synthesis of polysaccharides are disclosed.

A stepwise condensation of the monosaccharides and the groups A and B is possible. In general, however, building blocks consisting of D-glucose, L-idose, D-glucuronic acid or L-iduronic acid, suitably functionalized with the required alkyl, aryl, or aralkyl groups or by temporarily protective groups, are 40 condensed together in the desired order. In this way the (protected) trisaccharide unit can be prepared, which can be coupled with A and B, or protected derivatives thereof. It is also possible to condense a part of the trisaccharide unit with B or a protected derivative thereof, after which the compound obtained is coupled with the remaining part of the trisaccharide, which may already be provided with group A or a protected derivative thereof. If A is not yet attached to the remaining part of the trisaccharide, the 45 condensed saccharide obtained can be coupled to A (or a protected derivative thereof) in a manner known *per se*. In a similar manner, a part of the (protected) trisaccharide unit containing A can be coupled to the other part, which may be provided with B (or a protected derivative thereof), after which, if B is not present, the coupling product is condensed with B or a protected derivative thereof. Suitable protective groups are well known in carbohydrate chemistry. Preferred protective groups include benzyl and acetyl for hydroxy 50 groups, and methyl and benzyl for the carboxylate group of uronic acids. Other protective groups like levulinoyl, chloroacetyl, trityl, benzoyl, etc, may be used with equal success. Coupling of the saccharides is performed in a manner known in the art, e.g. deprotection of the 1-position of the glycosyl-donor, activation of this position (e.g. by making a bromide, pentenyl, fluoride or trichloroacetamide derivative) and coupling the activated glycosyl-donor with an optionally protected glycosyl-acceptor.

55 This process of stepwise or building block synthesis affords a protected carbohydrate derivative comprising a trisaccharide unit of the general formula:



10 in which R is alkyl, aryl, or aralkyl as previously defined, P<sub>1</sub> is an acid protective group (preferably methyl or benzyl), P<sub>2</sub> is a hydroxy protective group (preferably benzyl or acetyl), P<sub>3</sub> is a hydroxy protective group or the required alkyl, aryl, or aralkyl group, and A' and B' are A and B respectively, or protected derivatives thereof. Carbohydrate derivative IV can be deprotected and sulfated in a manner as described in the  
 15 previously mentioned EP 0,301,618, in order to obtain the carbohydrate derivative according to formula I. Suitable deprotection methods are, for example, basic hydrolysis for acetyl- and methyl-esters, and hydrogenolysis for benzyl ethers. Sulfation can successfully be performed with complexes of sulfur trioxide with bases like trimethylamine, triethylamine or pyridine in a suitable solvent.

For the treatment of venous thrombosis or for the inhibition of smooth muscle cell proliferation the  
 20 compounds of the invention may be administered enterally or parenterally, and for humans preferably in a daily dosage of 0,001-10 mg per kg body weight. Mixed with pharmaceutically suitable auxiliaries, the compounds may be compressed into solid dosage units, such as pills, tablets, or be processed into capsules or suppositories. By means of pharmaceutically suitable liquids the compounds can also be applied as an injection preparation in the form of a solution, suspension, emulsion, or as a spray, e.g. a  
 25 nasal spray.

The invention is further illustrated by the following examples.

#### Example 1

30 methyl 0-3,4-di-O-methyl-2,6-di-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl-2-O-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranoside undecakis sodium salt

a. methyl 6-O-acetyl-3,4-di-O-methyl-2-O-phenylmethyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-(methyl 2,3-di-O-methyl- $\beta$ -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)-O-3,6-di-O-acetyl-2-O-phenylmethyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-(methyl 2-O-acetyl-3-O-methyl- $\alpha$ -L-idopyranosyluronate)-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-acetyl- $\alpha$ -D-glucopyranoside (0,061 mmol; prepared according to the methods described in EP 84,999) was dissolved in 4,2 ml of chloroform and added to a mixture of 3 ml of chloroform, 18 ml of methanol and 3,7 ml of 4N sodium hydroxide. The mixture was stirred for 20 h at room temperature and after neutralizing with a diluted aqueous hydrochloric acid solution, evaporated to dryness. The residue was treated with methanol and the insoluble salts were filtered off. The filtrate was evaporated to dryness to obtain methyl 0-3,4-di-O-methyl-2-O-phenylmethyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O-2-O-phenylmethyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranoside disodium salt.  
 35 b. Crude methyl 0-3,4-di-O-methyl-2-O-phenylmethyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O-2-O-phenylmethyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranoside disodium salt was dissolved in a mixture of 3 ml of water and 1 ml of methanol and 50 mg of 10% Pd/C were added. The mixture was stirred under an atmosphere of hydrogen for 24 h at room temperature. After filtration the filtrate was evaporated to dryness, dissolved in water and desalting on Sephadex G-25. The combined fractions containing the pentasaccharide were evaporated to dryness to give methyl 3,4-di-O-methyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranoside disodium salt.  
 40 c. methyl 0-3,4-di-O-methyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranoside di sodium salt was dissolved in a mixture of 2,5 ml of dry dimethylformamide and 1,4 mmol triethylamine sulfurtrioxide complex. The mixture was stirred for 20 h at 50 °C, after which the mixture was cooled to room temperature and a mixture of 500 mg of sodium hydrogen carbonate in 7 ml of water was added.

The mixture was stirred for 30 min and then evaporated to dryness. The residue was dissolved in water, desalted on Sephadex G-25, after which the combined fractions were freeze-dried to give amorphous methyl 0-3,4-di-O-methyl-2,6-di-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl-2-O-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranoside undecakis sodium salt.  $[\alpha]_D^{20} = +51.3^\circ$  (c 0.4; water). Anomeric protons chemical shifts: 4.68; 5.14; 5.25; 5.28; and 5.40 ppm.

### Example 2

- 10 In a similar manner as described in Example 1 can be prepared:  
methyl 0-2,3,4,6-tetra-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl-2-O-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranoside tridecakis sodium salt.  $[\alpha]_D^{20} = +44.5^\circ$  (c 1; water). Anomeric protons chemical shifts: 4.52; 4.79; 4.97; 5.36; and 5.48 ppm.
- 15 methyl 0-2,3,4,6-tetra-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl-2-O-sulfo- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl-2-O-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranoside tetradecakis sodium salt.  $[\alpha]_D^{20} = +45.1^\circ$  (c 1; water). Anomeric protons chemical shifts: 4.76; 5.14; 5.15; 5.48; and 5.64 ppm.
- 20 methyl 0-3,4-di-O-tetradecyl-2,6-di-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl-2-O-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranoside undecakis sodium salt.  $[\alpha]_D^{20} = +39.0^\circ$  (c 1; water). Anomeric protons chemical shifts: 4.66; 5.09; 5.24; 5.47; and 5.57 ppm.
- 25 methyl 0-3,4-di-O-n-hexyl-2,6-di-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl-2-O-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranoside undecakis sodium salt.  $[\alpha]_D^{20} = +40.8^\circ$  (c 1; water). Anomeric protons chemical shifts: 4.68; 5.14; 5.16; 5.52; and 5.56 ppm.
- 30 methyl 0-3,4-di-O-methyl-2,6-di-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl-2-O-sulfo- $\beta$ -D-glycopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl-2-O-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranoside dodecakis sodium salt.  $[\alpha]_D^{20} = +38.5^\circ$  (c 1; water). Anomeric protons chemical shifts: 5.13; 5.14; 5.31; and 5.56 ppm.
- 35 methyl 0-[4-phenylmethoxycarbonylamino]phenyl]-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl-2-O-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranoside dodecakis sodium salt.  $[\alpha]_D^{20} = +56.0^\circ$  (c 1; water). Anomeric protons chemical shifts: 4.69; 5.16; 5.54; and 5.67 ppm.
- 40 methyl 0-(4-aminophenyl)-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-methyl-2-O-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranoside tridecakis sodium salt.  $[\alpha]_D^{20} = +57.0^\circ$  (c 1; water). Anomeric protons chemical shifts: 4.69; 5.13; 5.13; 5.53; and 5.67 ppm.

### Example 3

- 45 methyl 0-3,4-di-O-methyl-2,6-di-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-octyl-2-O-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranoside undecakis sodium salt.
- 50 a. methyl 0-6-O-acetyl-3,4-di-O-methyl-2-O-phenylmethyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-(phenylmethyl) 2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyluronate-(1 $\rightarrow$ 4)-O-3,6-di-O-acetyl-2-O-phenylmethyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-(phenylmethyl) 2-O-benzoyl-3-O-octyl- $\alpha$ -L-idopyranuronosyluronate-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-benzoyl- $\alpha$ -D-glucopyranoside (0.051 mmol) was dissolved in methanol and the catalyst (10% Pd/C) was added. The mixture was stirred under an atmosphere of hydrogen for 2 days at room temperature then, after filtration, the solvent was evaporated to dryness to give methyl 0-6-O-acetyl-3,4-di-O-methyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronate-(1 $\rightarrow$ 4)-O-3,6-di-O-acetyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2-O-benzoyl-3-O-octyl- $\alpha$ -L-idopyranuronate-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-benzoyl- $\alpha$ -D-glucopyranoside.

b. methyl 0-6-O-acetyl-3,4-di-O-methyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranonate-(1 $\rightarrow$ 4)-O-3,6-di-O-acetyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2-O-benzoyl-3-O-octyl- $\alpha$ -L-idopyranuronate-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-benzoyl- $\alpha$ -D-glucopyranoside was dissolved in methanol (8 ml) and sodium hydroxyde (5N solution) was added (0.9 ml). After 15 minutes at room temperature the solution was neutralized with Dowex-50-H<sup>+</sup> resin, filtered and evaporated to dryness to give methyl 0-3,4-di-O-methyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranonate-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-octyl- $\alpha$ -L-idopyranuronate-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranoside (48 mg).

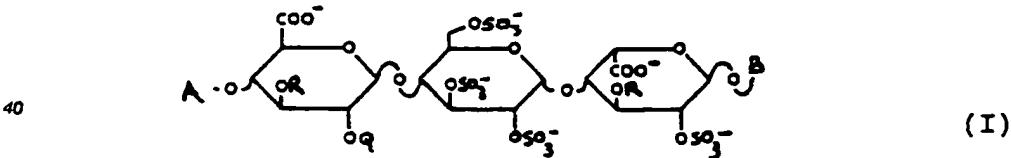
c. methyl 0-3,4-di-O-methyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranonate-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-octyl- $\alpha$ -L-idopyranuronate-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranoside (48 mg) was dissolved in dimethylformamide (1.4 ml) and sulfur trioxidetriethylamine complex (1.8 mmol) was added. After one night at 55 °C the mixture was cooled to room temperature and a mixture of sodium hydrogen carbonate (500 mg) and water (2 ml) was added. After evaporation the residue was layered on top of a Sephadex G25 column and eluted with water. The appropriate fractions were combined and freeze dried to give methyl 0-3,4-di-O-methyl-2,6-di-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2,3-di-O-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-3-O-octyl-2-O-sulfo- $\alpha$ -L-isopyranuronosyl-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-sulfo- $\alpha$ -D-glucopyranoside undekakis sodium salt.  $[\alpha]_D^{20} = +40^\circ$  (c 1.2; water). Anomeric protons chemical shifts: 4.68; 5.12; 5.13; 5.52; and 5.53 ppm.

### Example 4

20 In a similar manner, as described in Example 3, were prepared:  
methyl 0-3,4-di-0-methyl-2,6-di-0-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-0-2,3-di-0-methyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-0-2,3,6-tri-0-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-0-3-0-butyl-2-0-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-0-2,3,6-0-sulfo- $\alpha$ -D-glucopyranoside undecakis sodium salt.  $[\alpha]_D^{20} = +40^\circ$  (c 0.86; water).  
25 methyl 0-3,4-di-0-methyl-2,6-di-0-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-0-2,3-di-0-butyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-0-2,3,6-tri-0-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-0-3-0-octyl-2-0-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-0-2,3,6-tri-0-sulfo- $\alpha$ -D-glucopyranoside undecakis sodium salt.  $[\alpha]_D^{20} = +38^\circ$  (c 0.95; water).  
methyl 0-3,4-di-0-methyl-2,6-di-0-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-0-2,3-di-0-butyl- $\beta$ -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-0-2,3,6-tri-0-sulfo- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-0-3-0-methyl-2-0-sulfo- $\alpha$ -L-idopyranuronosyl-(1 $\rightarrow$ 4)-0-2,3,6-tri-0-sulfo- $\alpha$ -D-glucopyranoside undecakis sodium salt.  $[\alpha]_D^{20} = +43^\circ$  (c 0.92; water).

## **Claims**

1. A carbohydrate derivative comprising a trisaccharide unit of the general formula I

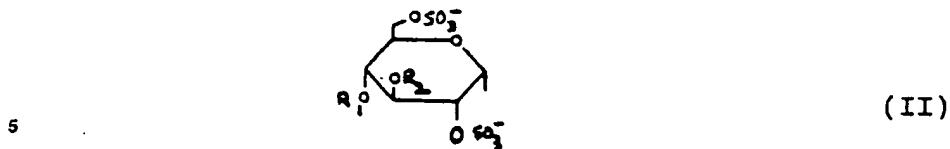


45 in which R is alkyl, aryl, or aralkyl;  
Q is alkyl, aryl, aralkyl, or  $\text{SO}_3^-$ ;  
A and B are independently selected from hydrogen, alkyl, aryl, aralkyl, or a carbohydrate group;  
and the charged moieties are compensated by counter-ions.

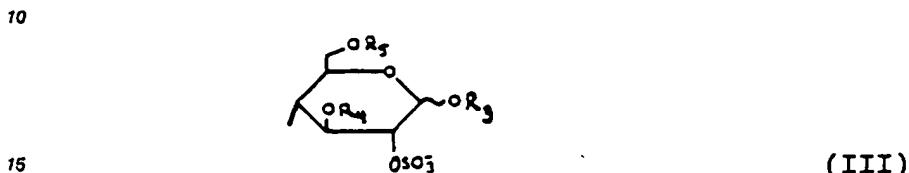
- 50 2. The carbohydrate derivative according to claim 1, characterized in that R represents methyl and Q represents methyl.

3. The carbohydrate derivative according to claim 1, characterized in that R represents methyl and Q represents  $\text{SO}_3^-$

55 4. The carbohydrate derivative according to claim 2 or 3, characterized in that A represents:

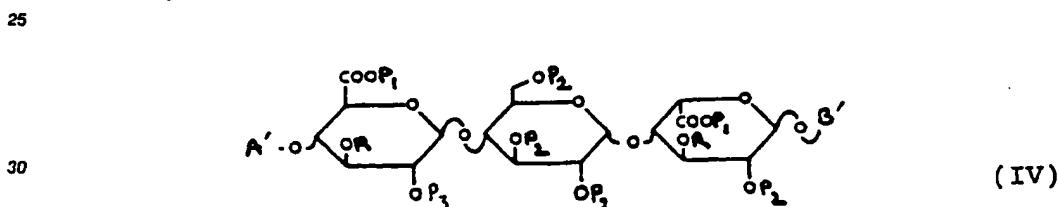


and B represents:



in which R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> are independently selected from hydrogen, alkyl, aryl, aralkyl, or SO<sub>3</sub><sup>-</sup>.

- 20 5. The carbohydrate derivative according to claim 4, characterized in that R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> are methyl, R<sub>4</sub> and R<sub>5</sub> are SO<sub>3</sub><sup>-</sup>, and OR<sub>3</sub> has the α-configuration.
6. A process for the preparation of the carbohydrate derivative of claim 1, characterized in that a protected carbohydrate derivative comprising a trisaccharide unit of the general formula:



35 in which R is alkyl, aryl, or aralkyl, P<sub>1</sub> is an acid protective group, P<sub>2</sub> is a hydroxy protective group, P<sub>3</sub> is a hydroxy protective group or an alkyl, aryl, or aralkyl group, and A' and B' are A and B respectively, or protected derivatives thereof, is deprotected and sulfated in a manner known in the art.

- 40 7. A pharmaceutical composition comprising the carbohydrate derivative of claim 1, admixed with pharmaceutically acceptable auxiliaries.
8. A use of the carbohydrate derivative of claim 1 for the manufacture of a medicament for the treatment of thrombosis and inhibition of muscle cell proliferation.

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REPORT

Application Number

EP 91 20 0895

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.5)		
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)		
A,D	EP-A-0 301 618 (AKZO) "Abstract; page 6, lines 16-19; page 7, lines 12-14; claim 1" -----	1,7,8	C 07 H 15/04 C 07 H 11/00 A 61 K 31/70		
TECHNICAL FIELDS SEARCHED (Int. Cl.5)					
C 07 H 15/00 C 07 H 11/00 A 61 K 31/00					
The present search report has been drawn up for all claims					
Place of search	Date of completion of search	Examiner			
The Hague	04 July 91	BRENNAN J.			
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